IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
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Mermod	)	Atty. Dkt. **3024-119**
	)	
Serial No. **National Stage of PCT/EP2004/011974**	)	Examiner: n/a
	)	
Filed: herewith	)	Group Art Unit: n/a

For: **HIGH EFFICIENCY GENE TRANSFER AND EXPRESSION IN MAMMALIAN CELLS BY A MULTIPLE TRANSFECTION PROCEDURE OF MAR SEQUENCES**

PRELIMINARY AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Please amend the above-identified application as delineated below.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 3 of this paper.

Remarks begin on page 13 of this paper.

Amendments to the Specification:

On page 1, between line 1 and 2, directly after the title, please insert the following paragraph:

-- This is the U.S. national stage of International application **PCT/EP2004/011974**, filed October 22, 2004 designating the United States and claiming the benefit of U.S. provisional application 60/513,574, filed October 24, 2003 and priority to European application EP04002722.9, filed February 6, 2004. --

On page 43, line 1 please delete “Claims” and insert therefore:

-- What is claimed is: --

Amendments to the Claims:

Please amend claims 1 to 10, 13 to 18, 20 to 34, 41 to 47, 49 to 60 and 62 and cancel claims 19, 35 to 40 and 61 as forth hereinafter.

Listing of Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended) A purified and isolated DNA sequence ~~having protein production increasing activity characterized in that said DNA sequence comprises~~ comprising

a) at least one bent DNA element,

b) and at least one binding site for a DNA binding protein, wherein said DNA sequence has protein production increasing activity.

2. (Currently Amended) The purified and isolated DNA sequence of claim 1 ~~characterized in that~~ wherein the bent DNA element contains at least 10% of dinucleotide TA, and/or at least 12% of dinucleotide AT on a stretch of 100 contiguous base pairs.

3. (Currently Amended) The purified and isolated DNA sequence of claim 2 ~~characterized in that~~ wherein the bent DNA element contains at least 33% of dinucleotide TA, and/or at least 33% of dinucleotide AT on a stretch of 100 contiguous base pairs.

4. (Currently Amended) The purified and isolated DNA sequence of ~~claims 1 to 2, characterized in that it comprises~~ claim 1 comprising a MAR nucleotide sequence ~~selected from the group comprising the sequences~~ , wherein the sequence is SEQ ID Nos 1 to 27, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof ~~and~~ or variants.

5. (Currently Amended) The purified and isolated DNA sequence of ~~claims 1 to 2, characterized in that it comprises~~ claim 1 comprising a cLysMAR element and/or fragment, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof ~~and~~ or variants.

6. (Currently Amended) The purified and isolated DNA sequence of claim 5, ~~characterized in that~~ wherein said part thereof is a nucleotide sequence selected from ~~the~~ B, K and F regions.

7. (Currently Amended) The purified and isolated DNA sequence of ~~claims 1 to 6, characterized in that~~ claim 1, wherein said DNA binding protein is a transcription factor.

8. (Currently Amended) The purified and isolated DNA sequence of claim 7, ~~characterized in that~~ wherein the transcription factor is ~~selected from the group comprising the~~ a polyQpolyP domain protein ~~proteins~~.

9. (Currently Amended) The purified and isolated sequence of claim 7, ~~characterized in that~~ wherein the transcription factor is ~~selected from the group comprising~~ SATB1, NMP4, MEF2, S8, DLX1, FREAC7, BRN2, GATA 1/3, TATA, Bright, MSX, AP1, C/EBP, CREBP1, FOX, Freac7, HFH1, HNF3alpha, Nkx25, POU3F2, Pit1, TTF1, XFD1, AR, C/EBPgamma, Cdc5, FOXD3, HFH3, HNF3 beta, MRF2, Oct1, POU6F1, SRF, V$MTATA_B, XFD2, Bach2, CDP CR3, Cdx2, FOXJ2, HFL, HP1, Myc, PBX, Pax3, TEF, VBP, XFD3, Brn2, COMP1, Evil, FOXP3, GATA4, HFN1, Lhx3, NKX3A, POU1F1, Pax6, TFIIA ~~and~~ or Vmw65 or a combination of two or more of these transcription factors.

10. (Currently Amended) A purified and isolated cLysMAR element and/or fragment having protein production increasing activity, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof ~~and~~ or variants.

11. (Currently Amended) The cLysMAR element and/or fragment of claim 10 consisting of at least one nucleotide sequence selected from ~~the~~ B, K and F regions.

12. (Original) A synthetic MAR sequence comprising natural MAR elements and/or fragments assembled between linker sequences.

13. (Currently Amended)The synthetic MAR sequence of claim 12, ~~characterized in that~~

wherein the MAR sequence comprises a cLysMAR element and/or fragment, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof ~~and~~ or variants.

14. (Currently Amended) The synthetic MAR sequence of ~~claims 12 to 13, characterized in that~~ claim 12, wherein the linker sequences are BglII-BamHI linker.

15. (Currently Amended) A method for identifying a MAR sequence using a Bioinformatic tool comprising ~~the~~ computing ~~of~~ values of one or more DNA sequence features corresponding to DNA bending,
major groove depth and minor groove width potentials, and
melting temperature.

16. (Currently Amended) The method ~~for identifying a MAR sequence using a Bioinformatic tool according to~~ of claim 15, ~~characterized in that~~ wherein said Bioinformatic tool contains algorithms, adapted to ~~the~~ use of profiles or weight-matrices, to compute values for one or more of said DNA sequence features corresponding to DNA bending, major groove depth and minor groove width potentials, and melting temperature.

17. (Currently Amended) The method ~~for identifying a MAR sequence using a Bioinformatic tool according to~~ of claim 16, ~~characterized in that~~ wherein said profiles or weight-matrices are based on dinucleotide recognition.

18. (Currently Amended) The method ~~for identifying a MAR sequence using a Bioinformatic tool according to~~ of claim 17, ~~characterized in that~~ wherein said Bioinformatic tool computes values for all of said DNA sequence features.

19. (Canceled)

20. (Currently Amended) ~~The method for identifying a MAR sequence using a Bioinformatic tool according to claims 15-19, characterized in that~~ The method of claim 15, wherein the ~~identification of~~ one or more DNA sequence features further ~~comprises~~ comprise a feature

corresponding to one or more binding sites for DNA binding proteins.

21. (Currently Amended) The method ~~for identifying a MAR sequence using a Bioinformatic tool according to~~ of claim 20, ~~characterized in that~~ wherein said DNA binding protein is a transcription factor.

22. (Currently Amended) The method ~~for identifying a MAR sequence using a Bioinformatic tool according to~~ of claim 21, ~~characterized in that~~ wherein the transcription factor is ~~selected from the group comprising~~ a polyQpolyP domain protein ~~proteins or transcription factors~~.

23. (Currently Amended) The method ~~for identifying a MAR sequence using a Bioinformatic tool according to claims 20 to 21, characterized in that~~ The method of claim 20 wherein the DNA binding protein is ~~selected from the group comprising~~ SATB1, NMP4, MEF2, S8, DLX1, FREAC7, BRN2, GATA 1/3, TATA, Bright, MSX, AP1, C/EBP, CREBP1, FOX, Freac7, HFH1, HNF3alpha, Nkx25, POU3F2, Pit1, TTF1, XFD1, AR, C/EBPgamma, Cdc5, FOXD3, HFH3, HNF3 beta, MRF2, Oct1, POU6F1, SRF, V$MTATA_B, XFD2, Bach2, CDP CR3, Cdx2, FOXJ2, HFL, HP1, Myc, PBX, Pax3, TEF, VBP, XFD3, Brn2, COMP1, Evil, FOXP3, GATA4, HFN1, Lhx3, NKX3A, POU1F1, Pax6, TFIIA ~~and~~ or Vmw65 or a combination of two or more of these transcription factors.

24. (Currently Amended) ~~The method for identifying a MAR sequence using a Bioinformatic tool according to claims 15-23, characterized in that~~ The method of claim 15, wherein values for ~~the identification of~~ identifying DNA bending ~~are comprised~~ comprise between 3 to 5 °.

25. (Currently Amended) The method ~~for identifying a MAR sequence using a Bioinformatic tool according to~~ of claim 24, ~~characterized in that~~ wherein values for the identification of DNA bending ~~are comprised~~ comprise between 3.8 to 4.4 °.

26. (Currently Amended) ~~The method for identifying a MAR sequence using a Bioinformatic tool according to claims 15-25 characterized in that~~ The method of claim 15, wherein values for the identification of the major groove depth ~~are comprised~~ comprise between 8.9 to 9.3 Å and values for the identification of minor groove width ~~are comprised~~ comprise between 5.2 to 5.8 Å.

27. (Currently Amended) ~~The method for identifying a MAR sequence using a Bioinformatic tool according to claims 26, characterized in that~~ The method of claim 26, wherein values for the identification of major groove depth ~~are comprised~~ comprise between 9.0 to 9.3 Å and values for the identification of minor groove width ~~are comprised~~ comprise between 5.4 to 5.7 Å.

28. (Currently Amended) ~~The method for identifying a MAR sequence using a Bioinformatic tool according to claims 15-27, characterized in that~~ The method of claim 16, wherein the melting temperature is ~~comprised~~ between 55 to 75 °C.

29. (Currently Amended) The method ~~for identifying a MAR sequence using a Bioinformatic tool according to~~ of claim 28, ~~characterized in that~~ wherein the melting temperature is ~~comprised~~ between 55 to 62 °C.

30. (Currently Amended) ~~The method for identifying a MAR sequence using a Bioinformatic tool of claims 15 to 29, characterized in that it further comprises~~ The method of claim 15 further comprising at least one filter predicting DNA binding sites for DNA transcription factors.

31. (Currently Amended) The method ~~for identifying a MAR sequence using a Bioinformatic tool according to~~ of claim 30, ~~characterized in that~~ wherein the filter is applied before or after the Bioinformatic tool.

32. (Currently Amended) The method according to ~~claims 30 to 31, characterized in that~~ claim 30, wherein the filter detects clusters of DNA binding sites using profiles or weightmatrices.

33. (Currently Amended) The method according to claim 32, ~~characterized in that~~ wherein the filter detects densities of clusters of DNA binding sites.

34. (Currently Amended) A method for identifying a MAR sequence ~~characterized in that it comprises~~ comprising
providing at least one filter detecting clusters of DNA binding sites, wherein said filter detects said clusters using profiles or weightmatrices.

35. to 40. (Canceled)

41. (Currently Amended) The purified and isolated DNA sequence of claim ~~40~~ 1, comprising a sequence selected from ~~the sequences~~ SEQ ID Nos 24 to 27, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof ~~and~~ or variants.

42. (Currently Amended) ~~The use of~~ A method for increasing protein production activity in a eukaryotic host cell comprising providing a purified and isolated DNA sequence comprising a first isolated matrix attachment region (MAR) nucleotide sequence, wherein the MAR nucleotide sequence is ~~which is a MAR nucleotide sequence selected from the group comprising~~:

- a purified and isolated DNA sequence of ~~claims 1 to 9~~ claim 1,
- ~~a purified and isolated MAR DNA of claims 35 to 41,~~
- one or more ~~the~~ sequences of SEQ ID Nos 1 to 27,
- a purified and isolated cLysMAR element and/or fragment,
- a synthetic MAR sequence of ~~claims 12 to 14~~ comprising natural MAR elements and/or fragments assembled between linker sequences,

a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof ~~and~~ or variants ~~for~~ increasing protein production activity in a eukaryotic host cell.

43. (Currently Amended) The ~~use of the purified and isolated DNA sequence~~ method of claim 42, ~~characterized in that~~ wherein said purified and isolated DNA sequence further comprises a promoter operably linked to a gene of interest.

44. (Currently Amended) ~~The use of the purified and isolated DNA sequence of claims 42 or 43,~~ ~~characterized in that~~ The method of claim 43, wherein said purified and isolated DNA sequence further comprises at least a second isolated matrix attachment region (MAR) nucleotide sequence which is a MAR nucleotide sequence ~~selected from the group~~ comprising:

- a purified and isolated DNA sequence of ~~claims 1 to 9~~ claim 1,
- ~~a purified and isolated MAR DNA of claims 35 to 41,~~

- one or more ~~the~~ sequences of SEQ ID Nos 1 to 27,
- a purified and isolated cLysMAR element and/or fragment,
- a synthetic MAR sequence of ~~claims 12 to 14~~ comprising natural MAR elements and/or fragments assembled between linker sequences,

a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof ~~and~~ or variants ~~for~~ increasing protein production activity in a eukaryotic host cell.

45. (Currently Amended) ~~The use of the purified and isolated DNA sequence~~ The method of claim 44, ~~characterized in that~~ wherein said first and at least second MAR sequences are located at both the 5' and the 3' ends of the sequence containing the promoter and the gene of interest.

46. (Currently Amended) ~~The use of the purified and isolated DNA sequence~~ The method of claim 44, ~~characterized in that~~ wherein said first and or at least second MAR sequences are located on a sequence distinct from ~~the one~~ a sequence containing the promoter and the gene of interest.

47. (Currently Amended) ~~The use of the purified and isolated DNA sequence of any of claims 42 to 46,~~ ~~characterized in that~~ The method of claim 42, wherein said purified and isolated DNA sequence is in ~~the~~ form of a linear DNA sequence as vector.

48. (Original) A method for transfecting a eukaryotic host cell, said method comprising
a) introducing into said eukaryotic host cell at least one purified DNA sequence comprising at least one DNA sequence of interest and/or at least one purified and isolated DNA sequence consisting of a MAR nucleotide sequence or other chromatin modifying elements,
b) subjecting within a defined time said transfected eukaryotic host cell to at least one additional transfection step with at least one purified DNA sequence comprising at least one DNA sequence of interest and/or with at least one purified and isolated DNA sequence consisting of a MAR nucleotide sequence or other chromatin modifying elements
c) selecting said transfected eukaryotic host cell.

49. (Currently Amended) The method of claim 48, ~~characterized in that~~ wherein said DNA sequence of interest is a gene of interest coding for a protein operably linked to a promoter.

50. (Currently Amended) The method of ~~claims 48 and 49, characterized in that~~ claim 48, wherein the selected transfected eukaryotic host cells are high protein producer cells with a production rate of at least 10 pg per cell per day.

51. (Currently Amended) The method of ~~claims 48-50, characterized in that~~ claim 48, wherein the MAR nucleotide sequence is ~~selected from the group comprising~~:

- a purified and isolated DNA sequence of ~~claims 1 to 9~~ claim 1,
- ~~a purified and isolated MAR DNA of claims 35 to 41,~~
- one or more ~~the~~ sequences of SEQ ID Nos 1 to 27,
- a purified and isolated cLysMAR element and/or fragment,
- a synthetic MAR sequence of ~~claims 12 to 14~~ comprising natural MAR elements and/or fragments assembled between linker sequences,

a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof ~~and~~ or variants.

52. (Currently Amended) The method of ~~claims 48-50, characterized in that~~ claim 48, wherein the MAR nucleotide is a purified and isolated sequence according to ~~claims 1 to 9~~ claim 1, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof and variants.

53. (Currently Amended) The method of ~~claims 48 to 52, characterized in that~~ claim 48, wherein the defined time corresponds to intervals related to the cell division cycle.

54. (Currently Amended) The method of claim 53, ~~characterized in that~~ wherein the defined time is ~~the~~ a moment the host cell just has entered into a second cell division cycle.

55. (Currently Amended) A method for transfecting a eukaryotic host cell, said method comprising co-transfecting into said eukaryotic host cell at least one first purified and isolated DNA sequence comprising at least one DNA sequence of interest, and a second and isolated

purified DNA comprising at least one MAR nucleotide selected from the group comprising:

- a purified and isolated DNA sequence of ~~claims 1 to 9~~ claim 1,
- ~~a purified and isolated MAR DNA of claims 35 to 41,~~
- one or more ~~the~~ sequences of SEQ ID Nos 1 to 27,
- a purified and isolated cLysMAR element and/or fragment,
- a synthetic MAR sequence of ~~claims 12 to 14~~ comprising natural MAR elements and/or fragments assembled between linker sequences,

a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof ~~and~~ or variants.

56. (Currently Amended) A process for the production of a protein wherein
a) a eukaryotic host cell transfected according to ~~claims 48 to 54 or~~ claim 55, is cultured in a culture medium under conditions suitable for expression of said protein and
b) said protein is recovered.

57. (Currently Amended) A transfected eukaryotic host cell comprising ~~transfected according to any one of claims 48 to 54 or claim 55~~ at least one purified DNA sequence comprising at least one DNA sequence of interest and/or at least one purified and isolated DNA sequence consisting of a MAR nucleotide sequence or other chromatin modifying elements.

58. (Currently Amended) A cell transfection mixture or kit comprising at least one purified and isolated DNA sequence according to ~~claims 1 to 9, 10 to 11, 12 to 14 or 35 to 41~~ claim 1.

59. (Currently Amended) A transgenic organism ~~characterized in that~~ wherein at least some of its cells have stably incorporated at least one DNA sequence of ~~claims 1 to 9, 10 to 11, 12 to 14 or 35 to 41~~ claim 1.

60. (Currently Amended) A transgenic organism ~~characterized in that~~ wherein its genome has stably incorporated at least one DNA sequence of ~~claims 1 to 9, 10 to 11, 12 to 14 or 35 to 41~~ claim 1.

61. (canceled)

62. (Currently Amended) A computer readable medium ~~characterized in that it comprises~~ comprising computer-executable instructions for performing the method for identifying a MAR sequence of ~~claims 15 to 33 and/or claim 34~~ claim 15.

Remarks

Claims 19, 35 to 40 and 61 are cancelled so that claims 1 to 18, 20 to 34, 41 to 60 and 62 are pending in this application of which claims 1, 10, 12, 15, 34 and 57 are in independent form. The above claim amendments are made to eliminate all multiple dependencies so as to reduce the filing fee, and also to eliminate improper multi-dependencies under U.S. practice so as to place the claims in form consistent with 37 CFR 1.75 (c) in order to insure examination of such claims.

The amendments are not "narrowing" amendments. The scope of the claims has not been reduced; no limitations have been added and none are intended.

The disclosure has been amended to add a priority claim and a more appropriate heading.

Respectfully submitted,

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